Performance Evaluation of the AnshLite Myelin Basic Protein Chemiluminescent Immunoassay Using Cerebrospinal Fluid

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Abstract

Background: Myelin is the insulating sheath surrounding neurons. Myelin basic protein (MBP) accounts for about one-third of total central nervous system (CNS) myelin protein. The cerebrospinal fluid (CSF) concentration of MBP increases in response to neuronal damage allowing the measurement of CSF MBP to be used clinically as a nonspecific marker of CNS inflammation. The objective of this study was to evaluate the performance characteristics of the AnshLite™ MBP chemiluminescent immunoassay (CLIA) for the quantitative determination of MBP in CSF.

Methods: MBP was measured using the AnshLite MBP CLIA (Ansh Labs, Webster, TX, USA). Performance characteristics including precision, linearity, analytical sensitivity, recovery, method comparison, MBP stability, the effects of freeze/thaw cycles, and the reference were evaluated using residual CSF specimens sent to ARUP Laboratories. The University of Utah's Institutional Review Board approved the project.

Results: Precision was determined using CSF pools with MBP concentrations of 3.51 and 1.54 ng/mL assayed in three replicates once each day for ten days. Within-run and total CVs were 9.6 and 14.8%, respectively. Linearity was determined by serially diluting a high MBP CSF sample with the zero calibrator to create six samples each tested in two replicates. The assay was linear within the measuring range to 11.25 ng/mL (linear regression $y=0.98(x)+0.05$, $R^2=1.00$). The limit of blank (LOB) and limit of detection (LOD) were determined by testing the zero calibrator ten times and 0.45 ng/mL calibrator seven times. The LOB was 0.01 ng/mL calculated as the mean concentration added to 3 SD. The LOD was 0.08 ng/mL calculated as the LOB added to 3 SD. Accuracy was determined by recovery studies performed by adding volumes of one of two calibrators (6 and 14 ng/mL) to two CSF samples with MBP concentrations of 1.18 and 0.34 ng/mL. Calculated recovery ranged from 86 to 109%. A method comparison using 42 samples with an MBP concentration range of 0.30-58.57 ng/mL was performed using the Beckman MBP ELISA (Beckman Coulter, Inc., Brea, CA) as the comparator method. Deming regression yielded $y=2.75(x)-2.68$, $R^2=0.95$, $Sx/y=4.78$. MBP stability was determined by storing two CSF specimens with MBP concentrations of 6.77 and 1.49 ng/mL at room temperature for two days, 4°C for two weeks, and -20°C for three weeks. MBP decreased <12% relative to time zero under all conditions. The effects of up to three freeze/thaw cycles were evaluated by testing two CSF samples with MBP concentrations of 7.02 and 1.59 ng/mL after each freeze/thaw cycle. The MBP concentration changed by -12.6, +6.94, and -18.0% relative to time zero after each cycle, respectively. The reference interval was established using 130 CSF specimens that were negative for oligoclonal bands. Using non-parametric analysis, this was determined to be less than 5.5 ng/mL.

Conclusions: The AnshLite™ MBP CLIA demonstrates acceptable performance characteristics for quantifying MBP in CSF. MBP is stable for two days at room temperature, two weeks at 4 °C and three weeks at -20 °C. Freeze/thaw cycles of CSF samples for MBP testing should be avoided.

Introduction

Myelin basic protein (MBP) accounts for about one-third of total central nervous system myelin protein. The cerebrospinal fluid (CSF) concentration of MBP increases in response to neuronal damage allowing the measurement of CSF MBP to be used clinically as a nonspecific marker of CNS inflammation. The objective of this study was to evaluate the performance characteristics of the AnshLite™ MBP chemiluminescent immunoassay (CLIA) for the quantitative determination of MBP in CSF.

Method

- MBP in CSF was measured by the AnshLite MBP CLIA kit (Ansh Labs, Webster, TX, USA), which is a quantitative sandwich immunoassay.
- Performance characteristics were evaluated using residual CSF specimens sent to ARUP Laboratories.
- Reference interval was established using 130 CSF specimens that were negative for oligoclonal bands.
- This study was approved by the University of Utah Institutional Review Board.

Results

Table 1. Precision was determined by measuring MBP at two concentrations using pooled patient samples in three replicates once each day for 10 days.

Mean concentration ng/mL	Repeatability CV, %	Between-day CV, %	Within-laboratory CV, %
3.506	8.0	10.1	12.9
1.538	9.6	11.2	14.8

Figure 1. Linearity was determined by serially diluting a high MBP CSF sample with the zero calibrator to create a set of six samples each tested in duplicate. The assay was linear within the measuring range of the calibrators to 11.25 ng/mL.

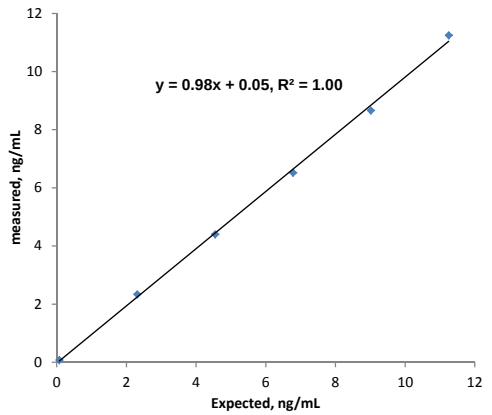


Table 2. Recovery was calculated by adding 10 µL of two concentrations of calibrators (6 ng/mL and 14 ng/mL) into 90 µL of one of two patient pools. Each sample was tested in duplicate.

Neat ng/mL	Spiking material ng/mL	Measured, ng/mL	Expected, ng/mL	Recovery, %
Patient pool	Cal 6	1.575	1.658	95.0
1.175	Cal 14	2.100	2.458	85.5
Patient pool	Cal 6	0.985	0.902	109.3
0.335	Cal 14	1.820	1.702	107.0

Table 3. Analytical sensitivity was studied as limit of blank (LOB) and limit of detection (LOD) by testing a blank Calibrator A (0 ng/mL MBP) in 10 replicates and Calibrator B (MBP concentration of 0.45 ng/mL) in 7 replicates. The LOB was calculated as the mean concentration of the blank added to 3 SD. The LOD was calculated as the LOB added to 3 SD of LOD.

	Mean, ng/mL	SD, ng/mL	Mean + 3SD, ng/mL
LOB	0.001	0.003	0.01
LOD	0.471	0.023	0.08

Table 4. MBP stability was determined by storing two CSF specimens with MBP concentrations of 6.77 and 1.49 ng/mL at room temperature for two days, 4°C for two weeks, and -20°C for three weeks. MBP decreased <12% relative to time zero under all conditions.

RT	Mean, ng/mL	% change	Mean, ng/mL	% change
t_0	7.02		1.59	
2 days	6.73	-4.13	1.59	0.32
4°C				
t_0	7.02		1.59	
2 weeks	6.46	-7.91	1.40	-11.67
-20°C				
t_0	6.52		1.39	
3 weeks	5.87	-10.05	1.33	-3.97

Table 5. The effects of up to three freeze/thaw cycles were evaluated by testing two CSF samples with MBP concentrations of 7.02 and 1.59 ng/mL after each freeze/thaw cycle. The percent changes in MBP concentration were calculated comparing to baseline.

	High level		Low level	
	MBP, ng/mL	% change	MBP, ng/mL	% change
Baseline	7.02	-----	1.59	-----
Freeze/thaw 1X	6.52	-7.06	1.39	-12.62
Freeze/thaw 2X	6.74	-3.99	1.70	6.94
Freeze/thaw 3X	6.38	-9.12	1.30	-17.98

Figure 2. The reference interval was established using 130 CSF specimens that were negative for oligoclonal bands. Using non-parametric analysis, an upper reference limit of 5.50 ng/mL (97.5%) was identified.

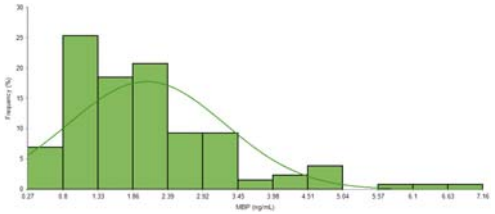
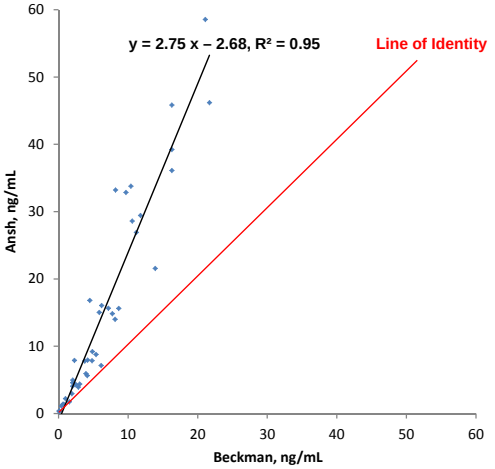


Figure 3. Method comparison of 42 patient samples in the range of 0.30-58.57 ng/mL was performed using the Beckman Coulter MBP ELISA assay as the comparator method. Deming regression and the correlation of the method comparison yielded: y = 2.75 x – 2.68, R^2 = 0.95. Because the AnshLite MBP CLIA detects almost all MBP fragments; higher concentrations compared to the Beckman Coulter assay are expected.



Conclusions

- The AnshLite MBP CLIA demonstrated acceptable performance characteristics for the quantitative determination of MBP in CSF.
- The assay was linear within the measuring range of the calibrators to 11.25 ng/mL.
- MBP was stable for 2 days at room temperature, 2 weeks at 4 °C, and 3 weeks at -20 °C.
- Freeze/thaw cycles of CSF samples for MBP testing should be avoided.
- The reference interval for this assay is less than 5.5 ng/mL.

Acknowledgement

Support for this study was provided by the ARUP Institute for Clinical and Experimental Pathology and AnshLabs provided all the kits.